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Report on the molecular characterisation of the genetic map of event Bt176

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1. Introduction: Bt176 maize dossier

Bt176 maize notification C/F/94/11-03 of Ciba-Geigy (Novartis) has been approved under Directive 90/220/EEC for growing, seed production, import, processing and food/feed purposes since 23 January 1997 (Commission Decision 97/98/EC).

The molecular data of the notification C/F/94/11-03 have been discussed during the meeting of the Belgium Biosafety Advisory Council on 17 February 2003. An overview of the molecular data of event Bt176 presented during this meeting and provided by different scientific institutions, namely CLO (Centrum Landbouwkundig Onderzoek, Melle, Belgium), JRC (Joint Research Centre, Ispra, Italy), TEPRAL (Strasbourg, France) and INRA (Institut National de la Recherche Agronomique, Versailles, France), and found in publications is given below. It must be noted that most of the data are preliminary and further research is needed for confirmation.

2. Overview on molecular data of event Bt176

2.1. Plasmids used for transformation

The event Bt176 maize was obtained by microprojectile bombardment into immature embryos of inbred corn line CG00526 (*Zea mays* L.) using two different plasmids (see annex 1). The plasmid pCIB4431 contains two copies of a synthetic truncated *cry1A(b)* gene, having approximately 65% homology at the nucleotide level with the native gene of *Bacillus thuringiensis* subsp. *kurstaki* strain HD1 (see fig. 1). The first copy is under the regulation of the maize phosphoenolpyruvate carboxylase (PEPC) promoter and the 35S terminator derived from cauliflower mosaic virus (CaMV). The second *cry1A(b)* copy is under the regulation of the maize calcium-dependent protein kinase (CDPK) promoter**,** resulting in pollen-specific expression, and the 35S terminator of CaMV. In addition, both *cry1A(b)* genes were combined with the intron #9 derived from the maize PEPC gene to enhance expression in maize. The plasmid pCIB3064 contains the *bar* gene derived from *Streptomyces hygroscopicus* under the regulation of the promoter and terminator sequence from the 35S transcript derived from CaMV (see fig. 2). Both plasmids also contain a copy of the *bla* gene, conferring resistance to ampicillin, under control of a bacterial promoter.

2.2. Characterisation of the insert and junction regions

There are still uncertainties about the copy number of the insert present in event Bt176. Koziel *et al*. (1993) indicated that there may be as many as five copies of the *cry1A(b)* genes present in Bt176. INRA provides results which suggest that at least 4 truncated inserts of the *bar* cassette are present (see annex 2). Quantitative studies of INRA on the p35S promoter suggest that 4 copies of p35S sequence are present which correlates with the suspected number of truncated *bar* cassettes (see annex 2).

Further studies on one of the inserts of the truncated *bar* cassette have been done (see annex 3). Investigations on the transfer of backbone sequences with the *bar* cassette revealed that pUC backbone sequences, corresponding to the origin of replication, adjacent to the 35S promoter were co-transferred (see fig. 3). PCR analysis together with sequence analysis of the 35S terminator border junction

showed that the 35S terminator is not inserted and that the *bar* coding sequence is immediately followed by maize plant DNA.

Sequence analysis of several parts of the inserts was done by the CLO (see annex 3). They proved that the *bar* coding sequence is similar to the sequence found in GENBANK accession n° X05822 (*Streptomyces hygroscopicus bar* gene), that the *cry* coding sequence showed 94% similarity with GENBANK accession n° AF537267 (synthetic construct *cryIAc* gene) and that the CDPK promoter is 100% similar to the sequence found in GENBANK accession n° L27484 (*Zea mays* CDPK gene).

2.3. Comparison of molecular data of Bt176 provided in dossier C/F/94/11-03 with data from other sources

Table 1: Comparison of molecular data of Bt176 according to dossier with data from other sources

Dossier	**Other sources**	**Remarks**
1st transgene insert (p35S-*bar*-t35S) present in at least 2 copies 5': no information 3': no information **2nd transgene insert** (t35S-int#9-*cryIA(b)*-pPEPC-pCPDK-*cryIA(b)*-int#9-t35S) present in 2 to 5 copies 5': no information 3': no information	**1st transgene insert** (p35S-*bar*-t35S) present in at least 4 truncated copies 5': pUC18 DNA possibly rearranged 3': t35S is not inserted, *bar* gene followed by maize plant DNA **2nd transgene insert** (t35S-int#9-*cryIA(b)*-pPEPC-pCPDK-*cryIA(b)*-int#9-t35S) present in at least 5 copies 5': no information 3': no information	Depending on the source, the number of truncated copies differs The 5' and 3' described here, are results obtained from one of the inserted *bar* cassettes

*CLO, JRC, TEPRAL, INRA and scientific publications (see references)

3. Conclusions

There are still uncertainties concerning the molecular data provided in the dossier C/F/94/11-03: it is not clear how many copies of each cassette are present. There are also indications that truncations of parts of the insert might have occurred. Therefore, the sequence of the insert should be further checked together with the number of inserts.

The molecular data presented in the dossier C/F/94/11-03 do not fulfil the Belgian requirements concerning molecular data. The sequence of the inserts, together with the DNA sequence of the flanking regions should be provided. In addition, bio-informatic analysis should be done to determine the presence of chimaeric open reading frames in the border integration sequences.

4. References

Koziel, M.G., *et al.* (1993). Field performance of elite transgenic maize plants expressing an insecticidal protein derived from *Bacillus thuringiensis*. *Bio/Technology* **11**, 194-200.

Smith, N., Kilpatrick, J.B., Whitelam, G.C. 2001. Superfluous transgene integration in plants. *Critical reviews in Plant Sciences* **20**, 215-249.

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5. Confidential information

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